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VARIABILITY AND AMPHIMIXIS

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A COMPARATIVE STUDY OF THE VARIABILITY IN ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) FORMED BY LATERAL (CLOSE BREEDING) AND BY SCALARIFORM (CROSS BREEDING) CONJUGATION, AND ITS BEARING ON THE THEORY OF AMPHIMIXIS AND CORRELATED PROBLEMS

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I. PRELIMINARY OUTLINE

1. *Introduction*

COMPARATIVE studies along statistical lines of the results produced by cross breeding and close breeding afford data of value bearing on the problem of evolution as well as the subsidiary problem of the origin of amphimixis. It has long been assumed (Weismann, '76) that sex existed primarily to increase variability and with the further assumption that the variations thus produced were heritable and accumulated, the differentiation of organisms was logically explained. As a corollary to such a conclusion the belief has long been prevalent that the offspring of organisms produced by cross breeding were as a group more variable than those produced by close breeding, an idea which gained further acceptance in connection with the investigations of Castle ('06), Jennings ('08, '09, '12, '13) and others interested in problems of genetics. That there was excellent evidence for exactly an opposite view and that an analysis of the results presented by the investigators mentioned above did not bear out the conclusion that variability was increased by cross breeding has been pointed out by the writer (Walton, '08, '12, '14) in some earlier papers.

The importance of arriving at a correct conclusion concerning the part played by hybridization and cross breeding in evolution can not be overestimated. If units are merely redistributed and form characters resulting in no actual evolutionary progress, work along Mendelian lines tends rather to obscure the facts of value toward solving the problem of the origin of species as well as that of evolutionary control in animal and plant breeding. It is therefore well to obtain data from as many sources as possible bearing on the question.

Among the species of *Spirogyra,* a group of algæ belonging to the class Conjugatæ, there are several which reproduce both by lateral conjugation (Fig. 1, *A*) where the adjacent cells of a single filament unite to form the zygospore, itself a young individual, and at the same time by scalariform conjugation (Fig. 1, *B*) where the cells of two distinct filaments unite to form the zygospore. Thus

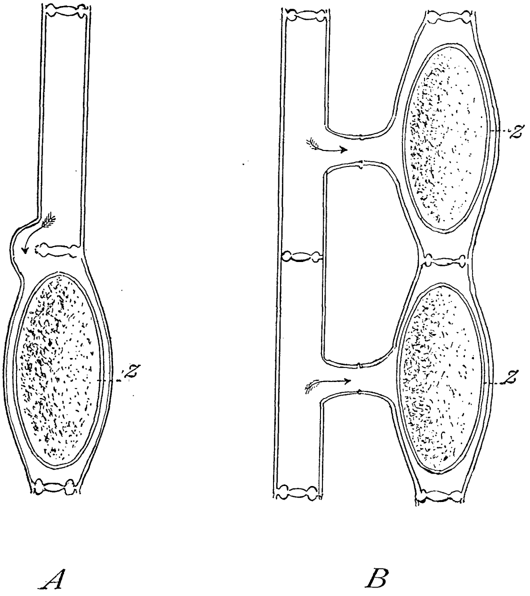

FIG. 1. FORMATION OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) by lateral conjugation (*A*) close bred from the same filament, and by scalariform conjugation (*B*) cross bred from two distinct filaments. *z* = zygospore.

there is an example of a population producing under the same environment two groups of individuals, one by close breeding (lateral conjugation) and the other by cross breeding (scalariform conjugation), and a comparison of the variability by statistical methods should afford evidence toward the solution of the problem presented where the offspring have arisen from a common ancestor as indicated in the material studied.

2. *Historical*

Much has been published concerning hybridization, cross and close breeding, amphimixis and parthenogenesis, all of which are distinguishable from one another merely by degree, nevertheless so far as the subject under discussion is concerned, the conclusions in general have largely been assumptions based on little or no evidence.

It was Weismann ('76) who was evidently the first to definitely express the importance of sex in producing variations, an idea to which he consistently held in his subsequent papers, while Nägeli ('84), Strasburger ('84), Hatscheck ('87), Haycraft ('95), etc., believed likewise on theoretical grounds that variability was reduced by amphimixis.

The first paper presenting tangible evidence upon the subject was that of Warren ('99) who found that parthenogenetically produced *Daphnia magna* were slightly more variable as measured by the "Standard Deviation" which had a value of 2.95, than the mothers whose "Standard Deviation" was 2.22. The small number utilized, 96 in the first instance and 23 in the second instance, together with the fact that the mothers represented a selected class, only those *Daphnia* producing young being included, did not allow placing much reliance in the results. Warren ('02) compared 60 parental aphids (*Hyalopterus trirhodus*) and their 368 offspring as well as a series from 30 aphid grandparents and their 291 grandchildren. The variability was found in a comparison of grandparents and grandchildren (parthenogenetic) to have slightly decreased in respect to frontal breadth and considerably increased in respect to length of right antenna, but again objections similar to those in the preceding paper render the conclusion of little value, as Warren himself observed.

Casteel and Phillips ('03) measured drones and workers of *Apis mellifica,* the honey bee, selecting individuals at random from different colonies, and tabulating classes and frequencies without, however, a further application of biometrical methods. The "range of variability" was found to be greater in the drones than in the workers. Lutz ('04) criticized the methods utilized in the paper, nevertheless variation as measured by the standard deviation upon calculation by Wright, Lee and Pearson ('07) was found greater in the drones by a difference ranging from 0.22 to 2.63 in respect to all five characters studied in the single group of 50 Italian workers and 50 drones of real value for comparative purposes.

Kellogg ('06), in a preliminary paper dealing with drones and workers of bees and also with female aphids, concluded that not only was there no evidence that amphimixis produced increased variability, but that it was an unnecessary factor in the production of Darwinian variation. The results were summarized as follows:

> (*a*) In all but one of the characteristics studied, the amount of variation both quantitative and qualitative, is markedly larger among the drone bees than among the workers, and in the one exceptional characteristic it is no less; (*b*) no more variation in wing characters is apparent among drones or workers that have not been exposed in imaginal condition to the rigors of personal selection than exists among bees, drones or workers, that have been so exposed; (*c*) the variation in wing characters in drone bees reared in worker cells is no greater than that among individuals reared among drone cells; (*d*) the variation among drones hatched from worker laid eggs is markedly larger than that among drones hatched from queen laid eggs. . . ."

Eleven "lots" were studied with a small number (No. 3, 48; No. 7, 54; No. 8, 75; No. 9, 26; No. 11, 60) in many of the "lots." Even though the probable errors would have been large and while the material was heterogeneous, the facts brought out are of extreme interest, particularly when considered with the results obtained by Casteel and Phillips ('03).

Wright, Lee and Pearson ('07) made a comparative biometrical study of 129 queens, 130 drones, and 129 workers taken from a nest of the common wasp *Vespa vulgaris* in Charterhouse, England. In connection with the wing dimensions, the coefficient of variation was found to be greatest in the worker, less in the drone, and least in the queen, differing from the bee as noted above where drones were more variable than workers. The conclusion here of interest was:

> There is no evidence in favor of parthenogenesis resulting in a smaller variability than sexual reproduction, for if the workers be more, the queens are less, variable than the drones.

It was suggested by the writers that the large variabilities of the workers might have resulted from subclasses among them due to differentiated functions or nurtures.

Castle, Carpenter, Clark, Mast and Barrows ('06) made observations on the variability and fertility of *Drosophila ampelophila* Loew, the small fruit fly, as modified by inbreeding and cross breeding. They found that "inbreeding does not affect the variability in number of teeth on the sex comb of the male, nor the variability in size." While the conclusion is not in accord with an earlier observation (p. 780) that variability would seem to have been increased by inbreeding so far as a comparison of the sixth inbred generation with the sixty-first generation, the small number utilized in the sixth generation (40 males in series *A*–6, *B*–6, *C*–6 each) was ground for the opinion that such a conclusion had little value in comparison with data pointing in the reverse direction. If however we calculate the coefficient of variation for the length of the tibia, an unfortunate omission on the part of the writers, it may be noted that the flies produced by inbreeding are decidedly more variable than those produced by cross breeding. Data for this conclusion are given in a subsequent part of the present paper.

Walton ('08) noted that the results of measuring zygospores of *Spirogyra* indicated that the close-bred individuals were more variable than the cross-bred individuals and furthermore that the data went far toward confirming the theory that sex existed for the purpose of limiting instead of augmenting variability.

Emerson ('10) found that crosses between races of plants (maize, squash, beans, gourds) differing in size and shape had the variability of the second (F_2) generation approximately twice as great as the variability of either parental form or of the first (F_1) generation. This he explained on the basis of the segregation of size and shape characters. Similar results were obtained by East ('11) for maize and Hayes ('12) for tobacco.

Jennings ('11) extending and summarizing his breeding experiments on Paramecium concluded that

> The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

Later ('13) continuing his investigations he stated that conjugation increased the variability in the rate of reproduction. In a subsequent part of the present paper a somewhat critical review of the data and conclusions therein noted is presented.

3. *Material*

In obtaining material early one April for the laboratory work of a class in biology, the collection being made in a small pool resulting from the overflow of a rivulet, a peculiar species of *Spirogyra* was noticed in which both lateral and scalariform conjugation was taking place often in the same filament. It was at once suggestive that a comparison of the variability in the two groups of zygospores would present facts of interest in connection with the effect of close breeding and cross breeding on variability as well as affording evidence as to the theories of amphimixis.

The species was first determined as *Spirogyra quadrata* (Hass.) but subsequent examination indicated that it should be classified as *Spirogyra inflata* (Vauch.).

The material utilized for the measurements was all procured at one time from a restricted area one or two inches square on the surface of the pool and included only the one form of *Spirogyra*, that alone being present as a mass 3 or 4 inches in diameter. Inasmuch as both lateral and scalariform conjugation occasionally took place in the same filament (Fig. 2) a suggestion that two species were represented can not be made for the filaments are alike in every characteristic. Of the 500 zygospores observed 45 per cent. were produced by lateral conjugation.

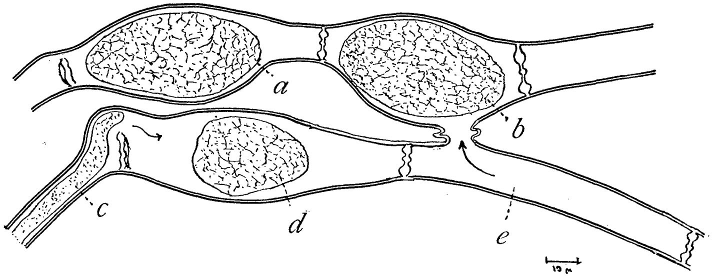

FIG. 2. *Spirogyra inflata* (Vauch.) × 800, with both scalariform and lateral conjugation in the same filament. (*a*) Zygospore formed by lateral conjugation. (*b*) Zygospore formed by scalariform conjugation. (*c*) Protoplasm of a "male" cell uniting with (*d*) the protoplasm of a "female" cell to form a zygospore by lateral conjugation. (*e*) Cell from which the protoplasm has passed to form the zygospore in (*b*). Obj. 1/12, Ocul. 2. Camera lucida drawing.

4. *Methods*

In considering the variability of large numbers of microscopic forms, rapid and accurate measurements are a necessity. Pearl and Dunbar ('03) in measuring *Arcella* used a camera lucida, marking the dimensions by means of a needle point, and reducing to microns. Pearl ('06) adopted a similar method for *Chilomonas,* using a magnification of 689.7. Pearl ('07) in measuring *Paramecium* used a 2/3-inch objective and a No. 1 ocular. By means of a camera lucida the points to be measured were projected on cards, marked, and measured with a vernier calipers to tenths of millimeters. Multiplying the measurements so obtained by the proper reduction factor found by calibrating with a stage micrometer, they were reduced to microns and recorded. Jennings ('11) at first measured *Paramecium* from a slide with an ocular micrometer. Later an Edinger drawing and projection apparatus was used, the projected images of the specimens on a slide in a flat drop of 25 per cent. glycerine, without a cover glass which by pressure would have caused distortion, were enlarged to 500 diameters and measured with a milimeter ruler.

In the present study, the material was preserved in 2 per cent. formalin, the first series of measurements[1] being made April 2, while measurements of 358 were completed before May 16, and the remaining 42 finished Aug. 21 of the same year. Swelling of the zygospores did not occur to an appreciable extent, a possible error considered in a subsequent part of the paper. Using a B. and L. BB-6 microscope with a No. 1 ocular and a 1/12 oil immersion, a slide with a couple of drops of fluid containing the material was covered with a No. 2 coverglass, the superfluous liquid drawn off by means of a pipette, and the preparation placed on the mechanical stage. Beginning at the lower right-hand corner the slide was moved from left to right and each zygospore presented in the field in a uniformly horizontal condition, was measured. On reaching the left margin of the preparation, the slide was returned to the first position, moved sufficiently toward the observer so that a new path would be traversed, and the operation repeated. Thus the selection was at random and no zygospore measured twice. The dimensions were marked on note paper by means of a camera lucida at a magnification of 1,460 diameters, the two cross lines representing length (x) and diameter (y) having at the point of juncture an "*S*" or an "*L*" for scalariform or lateral conjugation. Only those zygospores having definitely formed membranes were considered.

In the reduction of data, so soon as the projections of the apparent dimensions were completed, the length of the lines x and y were measured with proportional dividers (Keuffel and Esser No. 441 special) adjusted at the ratio 1,460 to 1,000, thus giving a reading in tenths of microns. Accurate adjustment was made possible by means of a micrometer screw, on the basis of the equation for similar triangles;

---

[1] I am indebted to Dr. C. C. W. Judd, of Baltimore, Md., at that time a senior in Kenyon College, for work in part in obtaining the first series of measurements.

$$1{,}460 \text{ mm.} : 1{,}000 \text{ mm.} = 160 \text{ mm.} - x \text{ mm.} : x \text{ mm.}$$

where 160 represented the total length of the dividers, and x or 65.04 mm. the point of adjustment. Having checked the adjustment, it only became necessary to note the size of a given zygospore with the longer legs of the instrument, then by applying the shorter legs to a millimeter scale, to read the result. The various constants were then computed on the basis of the work of Pearson and of Elderton by means of a Brunsviga calculating machine. I am indebted to Dr. H. H. Mitchell of the University of Illinois for checking the mathematical data.

II. CONSIDERATION OF RESULTS

The direct results obtained by the statistical methods employed are here presented. These furnish the basis for the general discussion and conclusions which follow. The problems of biology relating to evolution need the application of statistical methods to studies in genetics. In no other way will it be possible to clearly demonstrate the relative efficiency of the different types of variation—fluctuation, amphimutation, cumulation, etc.—in originating and maintaining the diverse forms of life that exist. Similarly the importance or unimportance of small variations in animal and plant breeding may only thus be explained. The refinements of curve fitting are by no means necessary, nevertheless values are thus exhibited which are presentable in no other way.

1. Comparative Variability in Length of Zygospores

In the frequency distribution for lengths of the two groups of zygospores (Table I) the class range adopted was two microns as compared with a range of one micron in the distribution of diameters. The more extended as well as the more irregular distribution of lengths of the lateral zygospores when compared with the scalariform zygospores is at once suggestive that the group thus close bred, is the more variable one. It is also of some interest to note that the empirical range of variation for the laterally formed zygospores,—with length from 49 m. to 83 m.,—is considerably greater than in the scalariform zygospores with lengths from 47 m. to 79 m. While this is not a measure of statistical variability, it undoubtedly has a genetic value.

TABLE I

Length of 400 Zygospores from *Spirogyra inflata* (Vauch.), 200 produced by Lateral Conjugation and 200 produced by Scalariform Conjugation, arranged in Classes according to Frequencies. Magnitudes in 1/10*m*

Length of zygospores in microns.

Class	Lateral Conjugation		Scalariform Conjugation	
	Frequency		Frequency	
	Observed	Calculated	Observed	Calculated
38.0–39.9	1	0.21	0	0.
40.0–41.9	0	0.43	0	0.
42.0–43.9	1	0.84	0	0.
44.0–45.9	3	1.49	0	0.
46.0–47.9	2	2.56	3	1.17
48.0–49.9	0	4.18	3	3.14
50.0–51.9	4	6.44	6	6.76
52.0–53.9	16	9.34	16	12.65
24.0–55.9	10	12.71	20	19.82
56.0–57.9	26	16.16	25	26.21
58.0–59.9	11	19.17	25	29.40
60.0–61.9	19	21.15	26	28.41
62.0–63.9	22	21.67	25	24.13
64.0–65.9	20	21.18	18	18.30
66.0–67.9	21	18.10	11	12.66
68.0–69.9	13	14.72	7	8.12
70.0–71.9	16	11.08	6	4.90
72.0–73.9	5	7.72	6	2.83
74.0–75.9	1	4.98	1	1.57
76.0–77.9	5	2.99	1	.85
78.0–79.9	2	1.66	1	.45
80.0–81.9	1	.86	0	0.
82.0–83.9	1	.42	0	0.
Total........	200	200.06	200	201.04

The general constants for the variability in the length of the zygospore of the two groups are shown below (Table II). It may be noted that the mean (M.) or aver-

TABLE II

GENERAL CONSTANTS FOR VARIATION IN LENGTHS OF ZYGOSPORES BASED ON 200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM CONJUGATION WITH A CLASS RANGE OF 2 MICRONS. STANDARD DEVIATION IN MICRONS

Constant		Lateral Conjugation (Close Bred)		Scalariform Conjugation (Cross Bred)	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number	N.	200	....	200	....
Mean	M.	62.38	±0.1776	60.44	±0.1345
Mode	Mo.	62.5123	....	59.5170	....
Standard deviation	σ.	7.4460	±0.1304	5.7474	±0.1104
Coefficient of variation	CV.	11.9364	±0.0330	9.5093	±0.0330
Skewness	Sk.	−.0356	±0.0468	.1589	± .0464

age length of the zygospores produced by lateral conjugation exceeds the mean of the scalariform conjugants by 1.94 microns, while the probable error for the first constant is ± .1776 and for the second constant ± .1345. The difference is therefore a significant one so far as the present material is concerned.

It is in the comparison of the standard deviations (σ) and the coefficients of variation (C. V.) that the results of most interest appear, however. The former constant in lateral conjugation has a value of 1.6986 in excess of the same constant in scalariform conjugation, or relatively 29 per cent. This is more than thirteen times the probable error. In the coefficient of variation, an abstract number permitting comparison with similar constants in other organisms, the results indicate that the variability in lateral conjugation exceeds that occurring in scalariform conjugation by 2.4271 or relatively 26 per cent., a result corroborated by the distribution of the diameters. The probable errors are sufficiently small in comparison with the differences noted, that they may be considered negligible.

Skewness is negative in the curve for lateral conjugation, the mean being on the left side of the mode, but its value is less than the probable error. In the curve for scalariform conjugation skewness is positive with a value slightly more than three times the probable error. Therefore the differences of the two constants appear to have no particular value so far as the present material is concerned.

The analytical constants (Table III) necessary for the fitting of the curves indicate that type IV curves may be used for each method of conjugation. In lateral conjugation the equation is

$$y = 10.842\left(1+\frac{x^2}{29.2687^2}\right)^{-33.0942} \times e^{-9.5995\tan -1(x/29.2687)}$$

and in scalariform conjugation similarly the equation is

$$y = 5.0014\left(1+\frac{x^2}{529}\right)^{-11.0945} \times e^{-8.9889\tan -1(x/11.50)}$$

while the frequency polygons and the fitted curves (Figs. 3, 4, and 5) illustrate the conditions diagramatically.

TABLE III

ANALYTICAL CONSTANTS FOR VARIATION IN LENGTH OF ZYGOSPORES FORMED BY LATERAL SCALARIFORM CONJUGATION.

Constant	Lateral Conjugation	Scalariform Conjugation
m_2	13.8606	8.2583
m_3	—3.9024	9.2989
m_4	606.8690	245.0223
B_1	.0057	.1535
B_1	.0756	.3918
B_2	3.1068	3.5928
k	.0219	.1654

---

[2] The following formulæ as the basis of the probable errors, may be noted:

$$PE_{\sigma} = .67449\sqrt{\frac{M_4 - M_2^2}{4M_2}} \Big/ n, \quad PE_{\mathrm{C.\,V.}} = \frac{.67449}{\sqrt{2n}}\left[1+2\left(\frac{v}{100}\right)^2\right]^{\frac{1}{2}},$$

$$PE_{\mathrm{Sk.}} = .67449\sqrt{\frac{3}{2n}} \Big/ \sqrt{1+3(\mathrm{Sk.})^2}.$$

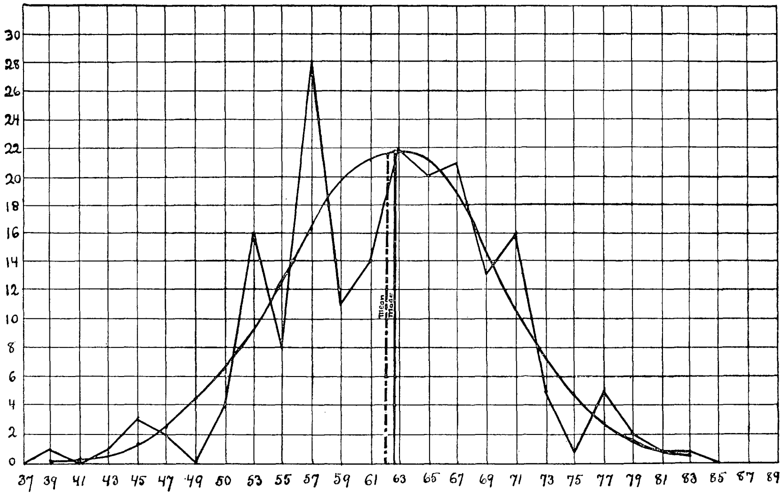

FIG. 3. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (INBREEDING) IN *Spirogyra inflata* (Vauch.).

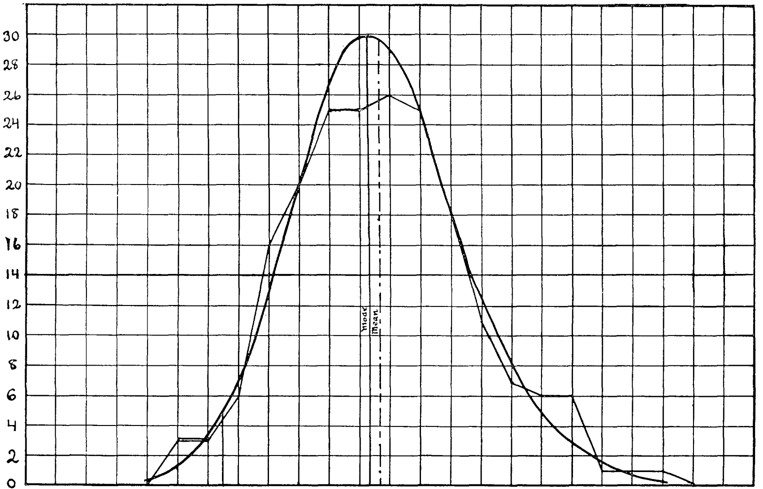

FIG. 4. FREQUENCY POLYGON AND FITTED CURVE FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.).

2. Comparative Variability in the Diameter of the Zygospores

The class range adopted in the frequency distribution for diameters (Table IV) of the two groups of zygo-

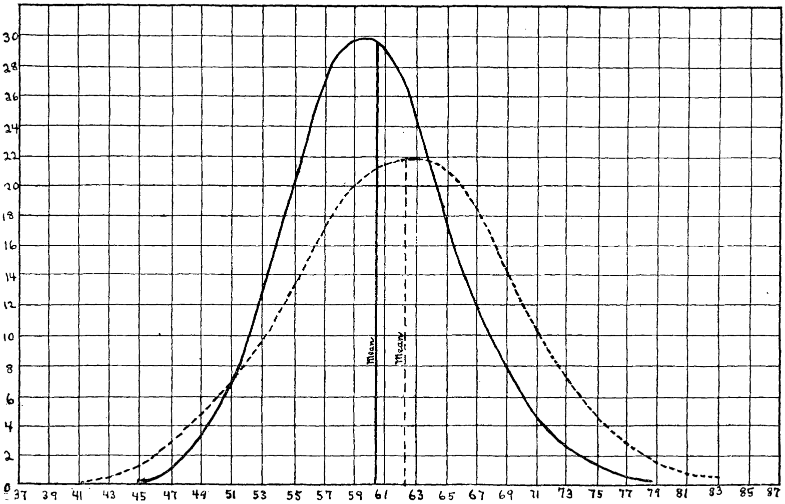

FIG. 5. COMPARISON OF FITTED CURVES FOR VARIATION IN LENGTH OF ZYGOSPORES PRODUCED BY LATERAL CONJUGATION (CLOSE BREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING) IN *Spirogyra inflata* (Vauch.). Lateral conjugation = — — — — — — —. Scalariform conjugation = ————————.

spores was one micron, measurement being made at the maximum diameter. An inspection of the distribution shows at once the greater concentration of the variates

TABLE IV

DIAMETERS OF 400 ZYGOSPORES FROM *Spirogyra quadrata* (HAAS.) 200 PRODUCED BY LATERAL CONJUGATION AND 200 PRODUCED BY SCALARIFORM CONJUGATION, ARRANGED IN CLASSES ACCORDING TO FREQUENCIES. MAGNITUDES IN 1/10 μ.

DIAMETER OF ZYGOSPORES IN MICRONS.

Lateral Conjugation		Scalariform Conjugation	
Class	Frequency	Class	Frequency
23.0–23.9	0	23.0–23.9	1
24.0–24.9	3	24.0–24.9	0
25.0–25.9	4	25.0–25.9	3
26.0–26.9	12	26.0–26.9	9
27.0–27.9	22	27.0–27.9	13
28.0–28.9	44	28.0–28.9	38
29.0–29.9	40	29.0–29.9	46
30.0–30.9	25	30.0–30.9	46
31.0–31.9	16	31.0–31.9	30
32.0–32.9	15	32.0–32.9	9
33.0–33.9	10	33.0–33.9	4
34.0–34.9	9	34.0–34.9	1
Total......	200	Total......	200

TABLE V

GENERAL CONSTANTS FOR VARIATION IN DIAMETERS OF ZYGOSPORES BASED ON 200 FORMED BY LATERAL AND 200 FORMED BY SCALARIFORM CONJUGATION WITH A CLASS RANGE OF 1 MICRON.

Constant		Lateral Conjugation, Close Breeding		Scalariform Conjugation, Cross Breeding	
Name	Symbol	Value	Prob. Error	Value	Prob. Error
Number	N.	200	....	200	....
Mean	M.	29.66	±.1049	29.725	±.0801
Mode	Mo.	29.1577	....	29.9735	....
Standard deviation	σ.	2.1980	±.0688	1.6796	±.0583
Coefficient of variation	C.V.	7.5376	±.0339	5.7471	±.0338
Skewness	Sk.	.2285	±.0505	−.1480	±.0566

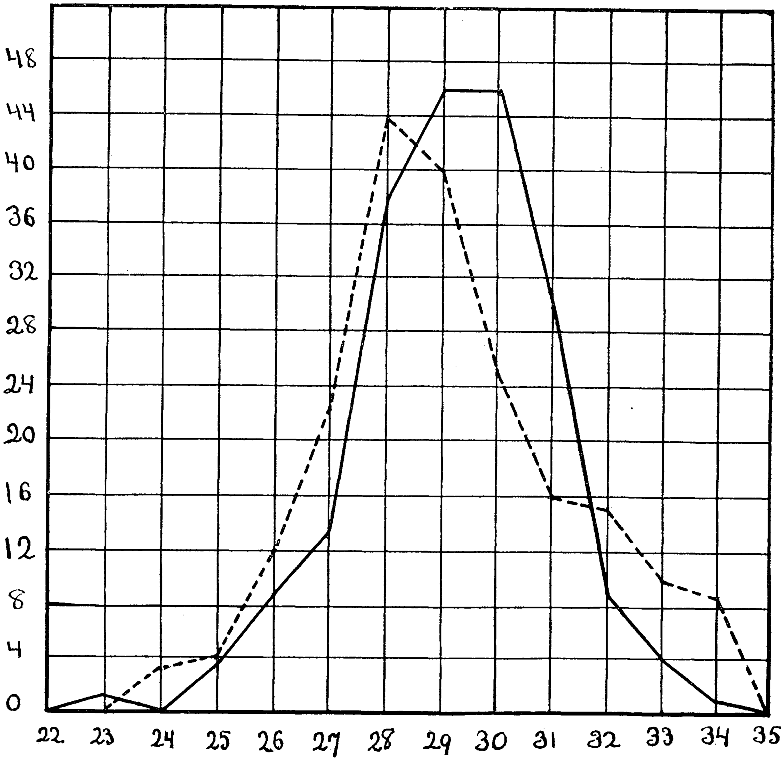

FIG. 6. DIAGRAM SHOWING THE FREQUENCY POLYGON FOR THE VARIATION IN DIAMETER OF ZYGOSPORES IN *Spirogyra inflata* (Vauch.) PRODUCED BY LATERAL CONJUGATION (INBREEDING) AND BY SCALARIFORM CONJUGATION (CROSS BREEDING). The unit of abscissa is one mikron. — — — — — — — = Lateral conjugation (close breeding). ———————— = Scalariform conjugation (cross breeding).

in scalariform conjugation, and thus their smaller variability. In considering the general constants of variability (Table V) based on the diameters it is to be noted that the means (M.) do not differ, as was found when considering length. The standard deviation (σ) and the coefficient of variation (C. V.) once more demonstrate the greater variability of the laterally formed zygospores. The values of the constants for skewness (Sk.) are not sufficient, however, when considered with the probable error, to be of importance. The frequency polygons (Fig. 6) illustrate conditions, although no curves have been fitted.

3. *Comparative Correlation of Length and Diameter*

In view of the results obtained in a consideration of the variability, it will be of some interest to ascertain whether

TABLE VI

CORRELATION BETWEEN LENGTH AND DIAMETER OF 200 ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) PRODUCED BY LATERAL CONJUGATION

Diameter *x* *y* Length	23.0–23.9	24.0–24.9	25.0–25.9	26.0–26.9	27.0–27.9	28.0–28.9	29.0–29.9	30.0–30.9	31.0–31.9	32.0–32.9	33.0–33.9	34.0–34.9	Totals
38.0–39.9	...	...	...	...	...	1	...	...	...	...	...	...	1
40.0–41.9	...	...	...	...	...	...	...	...	...	...	...	...	0
42.0–43.9	...	...	...	...	...	...	...	...	1	...	...	...	1
44.0–45.9	...	...	1	1	...	...	1	...	...	...	...	...	3
46.0–47.9	...	...	...	1	...	...	...	...	...	1	...	...	2
48.0–49.9	...	...	...	...	...	...	...	...	...	...	...	...	0
50.0–51.9	...	...	1	...	1	...	1	1	...	...	...	...	4
52.0–53.9	...	1	...	2	3	2	3	2	...	2	1	...	16
54.0–55.9	...	...	...	1	2	2	3	2	...	...	...	...	10
56.0–57.9	...	...	1	1	4	6	5	3	2	...	3	1	26
58.0–59.9	...	...	...	...	...	2	4	1	3	...	...	1	11
60.0–61.9	...	...	...	1	1	6	6	2	...	1	2	...	19
62.0–63.9	...	1	...	...	...	7	5	4	...	3	...	2	22
64.0–65.9	...	1	...	...	2	5	3	1	3	2	2	1	20
66.0–67.9	...	...	...	1	5	7	1	3	...	2	1	1	21
68.0–69.9	...	...	...	...	2	3	4	3	...	...	...	1	13
70.0–71.9	...	...	...	4	1	1	3	1	3	1	...	2	16
72.0–73.9	...	...	...	...	...	1	1	1	...	1	1	...	5
74.0–75.9	...	...	...	...	...	...	...	...	1	...	...	...	1
76.0–77.9	...	...	1	...	...	...	...	1	1	2	...	...	5
78.0–79.9	...	...	...	...	1	...	...	...	1	...	...	...	2
80.0–81.9	...	...	...	...	...	1	...	...	...	...	...	...	1
82.0–83.9	...	...	...	...	...	...	...	...	1	...	...	...	1
Totals........	...	3	4	12	22	44	40	25	16	15	10	9	200

the inbred zygospores produced by lateral conjugation will be more or less correlated than the cross bred zygospores produced by scalariform conjugation so far as length and diameter are concerned.

The value of perfect correlation as measured by the constant (r) is unity, while absence of correlation allows the value to become zero. Length is taken as the subject class (y) and diameter (x) as the relative class in the accompanying tables (VI and VII).

TABLE VII

CORRELATION BETWEEN LENGTH AND DIAMETER OF 200 ZYGOSPORES OF *Spirogyra inflata* (VAUCH.) PRODUCED BY SCALARIFORM CONJUGATION

Diameter x / y Length	23.0–23.9	24.0–24.9	25.0–25.9	26.0–26.9	27.0–27.9	28.0–28.9	29.0–29.9	30.0–30.9	31.0–31.9	32.0–32.9	33.0–33.9	34.0–34.9	Totals
38.0–39.9	...	...	...	...	...	...	...	...	...	...	...	...	0
40.0–41.9	...	...	...	...	...	...	...	...	...	...	...	...	0
42.0–43.9	...	...	...	...	...	...	...	...	...	...	...	...	0
44.0–45.9	...	...	...	...	...	...	...	...	...	...	...	...	0
46.0–47.9	...	...	...	...	1	...	1	...	...	...	1	...	3
48.0–49.9	...	...	...	...	...	1	...	1	1	...	...	...	3
50.0–51.9	...	...	...	2	...	2	1	1	...	...	...	...	6
52.0–53.9	1	...	...	1	1	2	5	4	2	...	...	...	16
54.0–55.9	...	...	...	2	2	4	6	4	1	...	1	...	20
56.0–57.9	...	...	1	...	2	5	5	5	4	3	...	...	25
58.0–59.9	...	...	...	2	...	4	5	13	...	...	...	1	25
60.0–61.9	...	...	1	...	...	1	9	7	7	1	...	...	26
62.0–63.9	...	...	1	...	2	6	6	3	6	...	1	...	25
64.0–65.9	...	...	...	1	2	3	4	2	4	2	...	...	18
66.0–67.9	...	...	...	...	1	3	1	3	1	1	1	...	11
68.0–69.9	...	...	...	1	...	2	1	1	2	...	...	...	7
70.0–71.9	...	...	...	...	1	3	1	...	...	1	...	...	6
72.0–73.9	...	...	...	...	...	1	1	1	2	1	...	...	6
74.0–75.9	...	...	...	...	...	...	...	1	...	...	...	...	1
76.0–77.9	...	...	...	...	...	1	...	...	...	...	...	...	1
78.0–79.9	...	...	...	...	1	...	...	...	...	...	...	...	1
Totals........	1	0	3	9	13	38	46	46	30	9	4	1	200

While one might infer that the longer a zygospore the greater the diameter, such a condition is not apparent by mere inspection of the tables in either case. Consequently on solving the equations we are prepared to find that the coefficients have an extremely low value in each group.

Lateral Conjugation .... r = .1894 ± .0460
Scalariform Conjugation .... r = .0934 ± .0473

Although in lateral conjugation the value is more than four times the probable error, one is scarcely prepared to state that there is greater correlation between characters in close breeding than in cross breeding on the basis of the data noted above. When considered with the results presented in Table XII, the conclusion seems fully established, however.

4. *Comparative Size of Zygospores*

The term "size" as noted in the subsequent discussion is open to various interpretations dependent as to whether length, diameter or volume is being considered, a condition which to some extent complicates the interpretation of size characters among multicellular organisms which are in general dependent on the number rather than the dimensions of the component cells.

Those zygospores produced by lateral conjugation (close bred), so far as the present material is concerned, have an average length considerably exceeding those produced by scalariform conjugation (cross bred) while the diameter is approximately the same. This is illustrated in Table VIII.

TABLE VIII

COMPARATIVE LENGTH, DIAMETER AND VOLUME OF ZYGOSPORES PRODUCED BY LATERAL (CLOSE BRED) AND BY SCALARIFORM (CROSS BRED) CONJUGATION

Method Produced	Mean Length	Mean Diameter	Mean Volume
Lateral conjugation........	62.380 m. ±.178	29.660 m. ±.105	28,733 cub. m.
Scalariform conjugation.....	60.440 m. ±.135	29.725 m. ±.080	27,972 cub. m.
Differences favoring lateral conjugation.............	+1.940 m.	−.070 m.	+771 cub. m.

Consequently, here, the average zygospore produced by lateral conjugation has a greater volume than that produced by scalariform conjugation. Utilizing the formula for computing the volume of a prolate spheroid ($V = 1/6\pi l d^2$) the difference is 771 cubic m. in favor of the former, although relatively this approximates only 3 per cent.

A question of some interest is at once suggested, namely, the possibilities for nourishment and development in cells of large and of small volume, inasmuch as one with a maximum volume has relatively less surface through which nourishment may be obtained. Thus growth may be retarded.

III. DISCUSSION OF RESULTS

The close bred forms on the basis of the characters studied in the given population have been found more variable as to both length and diameter, more highly correlated, and larger taking into consideration length and volume. The value of the conclusions in their application to the solution of problems of evolution is dependent on the logical application of cause and result as well as the methods of the investigation.

That the two groups of zygospores are comparatively close bred and cross bred will scarcely be denied, particularly when it is remembered that in lateral conjugation nearly all adjacent pairs of cells in a filament had united in the process, each pair producing a zygospore, all pairs having originated from the same cell. With the material taken from a part of a mass a few centimeters square, a sample of a whole population has been utilized, and from what is known of the reproduction of *Spirogyra*, it may be assumed with reasonable certainty that the entire mass had its origin from zygospores produced in a few filaments the preceding year. With practically all zygospores measured in each filament, the criticism that isolated zygospores of mixed descent were studied, and that greater variability would be expected in those produced by lateral conjugation, loses its force. Furthermore it is believed that all investigations thus far made, upon analysis support the direct conclusions which follow.

It may be objected that cells of mature filaments originating from the zygospores should have been studied. While this would have been of interest, the zygospores themselves are individuals in the cycle of development, and the differences as represented in the groups chosen can not be said to have less value than data from another part of the life cycle.

The possibility of the results being affected by the swelling of zygospores due to the 2 per cent. formalin used in preservation, became apparent when other duties prevented measurements within the anticipated time. The first series of 358 zygospores was measured between April 2 and May 16, while the remaining 42 were measured between August 17 and 21. The question seemed an important one, and in order to test the extent of such an error if present, the average diameter of the last lot was compared with that of the first lot, the values being 29.15 m. and 29.08 m., the difference of 0.07 m. being well within the limits of the probable error. The 42 zygospores measured August 17–21 happened to consist of an equal number of lateral and scalariform individuals, which would thus tend to eliminate an error should it have occurred. Consequently the use of the formalin does not appear to have affected the results.

Some evidence has been presented that new phylogenetic characters are more variable than older characters. Thus if lateral conjugation was a recent acquisition the greater variabitiy might have been expected. Pearl and Clawson ('07) found a higher variation in the great chela of the crayfish, *Camburus propinquus* Girard, than in the protopodites of the 2 and 3 legs, nevertheless they preferred to attribute the result to ontogenetic rather than to phylogenetic factors. MacDougall, Vail and Shull ('07) stated that

> the greater variability of phylogenetically new characters as compared with older ones . . . is confirmed . . . (p. 89).

The conclusion is open to objection inasmuch as they were comparing a hybrid with a single parental type and in general the greater variability would be expected. Consequently even admitting that lateral conjugation has been a more recent development than scalariform conjugation, it would not be demonstrated that an error had thus arisen.

1. *Comparative Variability*

Within the limits of the characters studied so far as the present material is concerned, it is evident that the zygospores produced by close breeding are more variable than those produced by cross breeding. While it is another proposition to extend the conclusion and insist that organisms produced asexually, by pure lines, or by close breeding, are more variable than those produced sexually or by cross breeding, it would seem that the facts strongly support such a conclusion and in connection with the evidence afforded by the investigations of Warren, Casteel and Phillips, Kellogg, and Wright, Lee and Pearson, it certainly may be denied that amphimixis or cross breeding as compared with other types actually produces variations, as has long been the prevalent belief.

The question here of particular interest, however, is that of the excess type of variability represented in *Spirogyra*. Inasmuch as the material was homogeneous in every way, it may be asserted that the greater variability exhibited by the close-bred forms is not fluctuability due to environment. It is also evident that, theoretically, cross breeding produces a greater number of combinations than inbreeding, nevertheless that the variability thus resulting is overwhelmed by that of another type in nature, is clear from the results noted in the preceding pages. An excellent demonstration of such condition is obtained by recalculating constants obtained by Hayes ('12) as shown in the accompanying table (Table IX) based on data obtained in connection with the breeding of *Nicotiana tabacum*.

TABLE IX

COMPARISON OF VARIABILITY OF *Nicotiana tabacum* IN COMBINED PARENTAL TYPES (NO. 3 AND 8) WITH VARIABILITY IN SEPARATE PARENTAL TYPES (NO. 1, 2, 6 AND 7), IN THE FIRST HYBRID GENERATIONS (NO. 4 AND 9) AND IN THE SECOND HYBRID GENERATIONS (NO. 5 AND 10). NO. 3 AND 8 CALCULATED FROM DATA BY HAYES IN TABLES NOTED. OTHER CONSTANTS AS GIVEN BY HAYES

No.	Table	Type	Character	S. D.	*E*	C. V.	*E*
1	XV	401	Number of leaves	0.96	±.037	5.00	±.189
2	XVI	403	" "	1.49	±.058	5.27	±.578
3	XV–XVI	401+403	" "	**4.70**	±.129	**19.55**	±.129
4	XVII	403×401	" "	1.30	±.056	5.51	±.215
5	XVIII	403×401−1−F_2	" "	2.24	±.103	9.40	±.551
6	XV	401	Height of plant	3.85	±.150	7.00	±.150
7	XVI	403	" "	4.55	±.177	5.98	±.177
8	XV–XVI	401+403	" "	**11.31**	±.311	**17.35**	±.312
9	XVII	403×401	" "	4.54	±.177	6.41	±.249
10	XVIII	403×401−1−F_2	" "	7.22	±.333	13.60	±.333

Here the constants of No. 3 and No. 8 have been obtained by combining the two parental types (401 and 403) both for the number of leaves and the height of the plant, and it may be noted that the coefficient of variation has dropped from 19.55 to 9.40 in the one case and from 17.35 to 13.60 in the other case. Thus variability as measured statistically has decreased. Those who have advocated an increased variability as the result of hybridization are correct when comparison is made of the F_2 generation with the F_1 generation or with a single parental generation. They are not correct, however, in making a general statement that cross breeding increases variability since the variability of the group composed of both parental types must be considered and upon so doing, it may normally be found that there has actually been a decrease in variability.

The possibility exists however that the variability will appear to have been increased when forms having the same phenotype but different genotypes are bred together. Such a condition may be illustrated by the two strains of white sweet peas crossed by Bateson which produced purple flowers in the first (F_1) generation, and purple, pink, mixed and white flowers in the second (F_2) generation. New combinations had arisen, but only as an expression of that which already existed in the phenotypes, for there is no evidence of an increase in unit characters nor was there an actual increase in variability.

There are only three papers of a statistical nature in which it has seriously been asserted that cross-bred forms or conjugating forms produced greater variability than resulted in close-bred forms or non-conjugating forms.

The first is that of Castle, Carpenter, Clark, Mast and Barrows ('06) based on a series of observations as to the effect of cross breeding and close breeding on the variability and fertility of the small fruit fly *Drosophila ampelophila* Loew. In conclusion it was stated that "inbreeding did not affect the variability in the number of teeth of the sex comb of the male, nor the variability in size," the first opinion resulting from the value of the coefficient of variation in the number of tibial spines, the second from the standard deviation in the length of the tibia. In the former case the data certainly do not permit a clear conclusion one way or the other. In the second case, however, if the value of the coefficient of variation is computed for the length of the tibia—which, strange to say, was not done in the original investigation—and thus allowance made for the greater length of

TABLE X

ILLUSTRATING COMPARATIVE VARIABILITY OF CROSS BRED AND INBRED FORMS OF *Drosophila* AFTER COMPUTING THE VALUE OF THE COEFFICIENT OF VARIATION FOR THE LENGTH OF TIBIA FROM DATA BY CASTLE AND OTHERS

S.D. = Standard Deviation. C.V. = Coefficient of Variation.

Group		Spines of Sex Comb		Length of Tibia	
Generation	Number	S. D.	C. V.	S. D.	C. V.
Cross bred (*X*–1)	100	1.749 ± .083	16.23	1.461 ± .070	**3.531 ± .168**
Inbred (*M*–31)	100	1.568 ± .075	15.51	1.723 ± .082	**4.452 ± .212**
Inbred (*N*–30)	100	1.684 ± .080	17.38	2.842 ± .136	**8.167 ± .389**
Inbred (*A*–61)	100	1.857 ± .089	17.60	2.041 ± .097	**5.245 ± .250**

tibia in the cross-bred forms (Table X) the average variability of the three inbred groups is 68 per cent. greater than that of the cross-bred group. Consequently, the results decidedly support the facts in the present paper.

The remaining papers are those of Jennings ('11 and '13) in a study of *Paramecium.* In the first paper the breeding experiments are summarized as follows:

> The progeny of conjugants are more variable, in size and in certain other respects, than the progeny of the equivalent non-conjugants. Thus conjugation increases variation.

It seems difficult to account for this conclusion if one subjects the data to a critical review. So far as a "pure race" is concerned the non-conjugants and their progeny were decidedly *more variable* than the conjugants and their progeny (Table 28, p. 94), although the small number utilized March 31 for the statistical work (42 and 34) is not sufficient to justify a conclusion in either direction. Even in a "wild culture" (Table 32, p. 99) the evidence is too conflicting to justify a definite expression of opinion. Of the seven comparisons here made among the progeny, five showed an excess variability for the conjugants, but in only one case did the difference exceed three times the probable error, while two cases showed an excess variability for the non-conjugants, the difference in one case exceeding twice the probable error. Data from numbers so small (22–95) can scarcely be considered reliable. The comparison of the variability of "all pairs" and "all unpairs" on June 22 and June 23 denotes an excess variability for those completing conjugation at the beginning of the experiment.

In the second paper Jennings concluded (p. 363) that conjugation increased the variation in the rate of reproduction. The variation was increased, but the explanation of such increase seems comparatively simple when it is noted that among the conjugants there were many with a low rate of fission with death occurring. As compared with the more normal rate of fission among nonconjugants, this could result in nothing but an increased variability, having, however, no bearing on the question at issue.

At the present time, therefore, it would seem that the preponderance of evidence demonstrates that variability is decreased in cross breeding.

2. *Comparative Size*

The zygospores produced by close breeding have a mean length of 62.38 μ. ± .18 μ. with a mean diameter of 29.66 μ. ± 10 μ. and those produced by cross breeding have a mean length of 60.44 μ. ± .13 μ. with a mean diameter of 29.725 μ. ± .08 μ. Thus so far as length is concerned the close bred zygospores are relatively 3.2 per cent. larger and although slightly smaller in diameter, when volume is considered by utilizing the formula ($V=\frac{1}{6}\pi l d^2$) the close bred forms are also 2.8 per cent. larger. Since these results are not in accord with the general belief that cross fertilization increases size and vigor, terms which have a diverse usage, however, it will be well to consider other evidence bearing on the problem with a view of attempting an explanation which may meet the conditions imposed.

Pearl ('07) in studying the conjugation of *Paramecium* with particular reference to assortative mating, notes that "the conjugant individuals when compared with the non-conjugant, are shorter and narrower" and stated in accordance with Calkins ('02) that the reduction in size was quite probably dependent on functional changes connected with reproduction. In *Spirogyra,* however, both the close-bred and the cross-bred zygospores go through similar reproductive processes in consequence of which one may question the theory that the method of conjugation is the decisive factor in bringing about the result even in *Paramecium.*

Jennings ('11) in comparing the size of conjugant and non-conjugant *Paramecium* stated that

> The progeny of conjugants . . . were a little larger than the progeny of non-conjugants and the difference appears to be significant.

This conclusion was based on measurements of length and diameter, the volume not being computed. When this is done as shown in the accompanying table (Table XI) by

TABLE XI

COMPARISON IN SIZE OF CONJUGANT AND NON-CONJUGANT FORMS OF *Paramecium aurelia* AND THEIR PROGENY BASED ON VOLUME $(V = 1/6_{\pi}ld^2)r$ FROM LENGTH AND DIAMETER MEASUREMENTS BY JENNINGS, 1911

Experiment			Non-conjugants and Progeny				Conjugants and Progeny				Non-conj. Exceed the Conjugants in Volume
Culture		Date, 1908	No.	Length Microns	Diam. Microns	Volume Cub. Microns	No.	Length Microns	Diam. Microns	Volume Cub. Microns	
	$Nf_?$	Mar. 31	34	144.59	34	**87,518**	48	136.95	35.52	**90,471**	− 2,953
a	Table	Apr. 10	65	137.97	44	**139,859**	61	148.20	42.30	**138,844**	+ 1,015
	28	Apr. 20	103	156.48	43.82	**157,327**	108	160.85	42.04	**148,849**	+ 8,478
	C_2	Sept. 16	110	132.18	?	?	138	121.91	?	?	+ ?
b	Table	Sept. 18	70	116.17	31.20	**59,211**	15	128	31.20	**65,241**	− 6,030
	29	Sept. 26	52	122.15	34.81	**77,500**	11	112.36	29.50	**51,198**	+ 26,302
	g	Sept. 27	118	135.35	?	?	174	118.28	?	?	+ ?
c	Table	Sept. 29	10	156.40	49.60	**201,465**	6	135.33	36	**91,833**	+109,633
	30	....	...	......	.....	......	...	......	......	......	+
	k	Sept. 12	100	140.20	?	?	336	129.58	?	?	?
d	Table	Oct. 28	10	136	37.60	**100,673**	39	131.38	35.49	**86,644**	+ 14,029
	31	Oct. 30	28	123.71	34.14	**75,497**	25	128.16	36.32	**88,520**	− 13,023

utilizing the formula $V = 1/6\pi ld^2$, thus allowing for slight decreases in diameters, the facts present a different interpretation.

Three (*a, b, c*) of the four experiments dealing with a "pure race" of *P. aurelia* indicate that the progeny of the non-conjugants become larger, even when as a group they are smaller (*a, b*?) at the beginning of the experiment. While the fourth (*d*) indicates a reverse condition so far as the measurements of October 30 are concerned, the measurements of the sixth and seventh generations immediately preceding, demonstrate that the non-conjugants were larger. The result on October 30, where the non-conjugants became smaller, may have depended on the elimination suggested by "all existing progeny." The extraordinary diminution in length (140 μ. to 123.71 μ.) suggests some disturbing factor of metabolism.

The results of the experiment with a "wild culture" where progeny of "unpaired" and "paired" forms of *P. caudatum* (?) were considered, again suggested to Jennings the greater size of the progeny of the paired individuals (conjugants), a condition which was particularly evident in the first generation. But it must be noted that the disturbance of the function of conjugation in "unpairing" may have produced the result. The progeny of the "unpairs" were relatively becoming larger from the first to the seventh generation. These facts taken together with the absence of measurements of mean diameters by which to calculate the mean volumes, suggest that such a conclusion based on that part of the work could not be accepted, and that the data strongly support the proposition directly contrary to Jennings that the progeny of conjugants tend to become smaller than the progeny of non-conjugants although the latter may be larger directly after conjugation as a result of slower fission. Thus the evidence from various sources, although incomplete, suggests that cross-bred unicellular organisms are smaller than close-bred forms.

Among multicellular organisms however it has long been recognized that hybrids usually grew to a larger size than either parental form, as has been observed by Kohlreuter ('63), Knight ('99), Gärtner ('49), as well as Darwin, Mendel and others, although the cause of the increased growth has been purely conjectural. It is quite evident that the result is due to either the increased number of cells, a suggestion made by East, to the increased size of the cells, or to the combination of both conditions.

The question immediately arises as to the cause of the increased size and vigor among cross-bred multicellular organisms when the evidence indicates that cross-bred unicellular organisms are smaller instead of larger.

Some investigations in progress[3] suggest an answer meeting the conditions, although more than a provisional opinion may as yet not be ventured. This is to the effect that the cells of cross-bred multicellular organisms are actually smaller than the cells of pure line or inbred organisms, and that the more rapid division is a function of the greater ratio surface has to volume in a small cell with the better opportunity this afforded for an increased metabolism.

The increase of size in plant and animal forms to the physiological limit has great importance for the future of agriculture and stock breeding, but many subsidiary problems must be solved before practical results are attained in this direction. The relative rate of growth, number and size of the constituent cells of pure line and of hybrid individuals is one of the problems.

3. Comparative Correlation Resulting from Close Breeding and Cross Breeding

The close-bred zygospores are more correlated as to length and diameter than the cross-bred zygospores, but since the difference only slightly exceeds twice the probable error, the value of the result here is questionable. Considering other investigations (Table XII), it may be noted that the group containing close-bred, asexual or non-conjugating organisms, is more highly correlated in respect to characters than the group consisting of cross-bred, sexual, or conjugating organisms, although two exceptions, No. 12 and No. 14, are presented. An interesting fact, although possibly only a coincidence, is that cross bred zygospores of *Spirogyra* and of conjugating *Paramecium* have approximately only one half the correlation exhibited by close bred zygospores of *Spirogyra* and by non-conjugating *Paramecium.*

The explanation of the conclusion here reached, that the value of a character "*x*" in cross-bred forms does not have the same tendency to change that the value of a

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[3] Walton ('14).

TABLE XII

COMPARATIVE CORRELATION OF CHARACTERS IN CROSS-BRED AND CLOSE-BRED ORGANISMS INCLUDING CONJUGANT AND NON-CONJUGANT *Paramecium*, AND PARTHENOGENETIC AND SEXUALLY PRODUCED WASPS

	Organism	Authority	Characters Compared	Type of Development	Value of "r"
1	*Paramecium*	Pearl, '07	Length and diameter.	Non-conjugants, Ser. *A*	.589±.03
2	"	" "		Conjugants.	.278±.04
3	*Drosophila*	Barrows, '06	Number of spines and length of tibia.	Close bred, Ser. *A*–61.	.469±.05
4	"	" "		" " Ser. *M*–31.	.448±.05
5	"	" "		" " Ser. *N*–30.	.708±.03
6	"	" "		Cross bred, Ser. *X*–1.	.141±.07
7	*Nicotiana*	Hayes, '12	Number of leaves and height.	Close bred, No. 401.	.368±.05
8	"	" "		" " No. 403.	.631±.03
9	"	" "		Cross bred, 403×401.	.406±.05
10	"	" "	Length and breadth of leaf.	Close bred, No. 401.	.684±.03
11	"	" "		" " No. 403.	.497±.04
12	"	" "		Cross bred, 403×401.	.818±.02
13	*Vespa vulgaris*	Wright, Lee, and Pearson, '07	Length and breadth of r. wing.	Drones.	.772±.02
14	"			Workers.	.912±.01
15	"			Queens.	.558±.04
16	*Spirogyra*	Walton, '15	Length and diameter.	Close bred (Lat. *C.*)	.189±.05
17	"	" "		Cross bred (Scal. *C.*)	.093±.05

related character "*y*" has in close-bred forms, apparently rests on a Mendelian basis. Its importance in evolution, beyond the idea that more pronounced temporary combinations are thus allowed in the trial and error plan of nature, is conjectural.

4. *Amphimixis and Death*

With the assumption that the results obtained in the preceding investigation, together with the data presented by other writers, when correctly analyzed, strongly supports the view that asexually produced organisms tend to be more variable than those produced by the union of two gametes, there is furnished evidence for the interpretation of the origin of sex—amphimixis and also for the origin of death that would seem to rest upon a much more secure basis than the purely speculative theories of Weismann, Nägeli, Hatscheck, Metschnikoff, Minot, etc., which have previously been advanced.

The chief advantage gained in the reduction of variability, while somewhat conjectural, would appear to be that of holding organisms within limited bounds, or in other words, *asexually produced organisms in general tend by their variability to exceed the limits of their environment and thus perish, while organisms produced by the mingling of two diverse lines of germ plasm with their lessened variability meet the conditions of the comparatively slowly changing environment and their race persists.* This idea was proposed entirely upon speculative grounds by Hatscheck ('87) who suggested that variation would run riot if not controlled by the union of germ cells, and it would now appear that the facts support such a proposition. While it has been suggested that the chief function of amphimixis was that of rejuvenation, a consideration of the discussion on "Comparative Size" as well as the recent experimental results obtained in the production of *Paramecium* do not support such an opinion to the exclusion of the hypothesis here put forward. East and Hayes ('12) have advanced the theory that recombinations in accordance with Mendelian principles were the chief purpose of amphimixis. While new combinations are thus brought about, apparently there exists a real difficulty in understanding how transitory heterozygotic forms could become of selective value in originating and maintaining such a process.

The acceptance of the conclusion that asexually produced organisms are more variable than those produced by amphimixis, and that thus some of the units are more readily subject to the eliminating influences of the environment, affords a comparatively simple explanation of the origin of death in multicellular forms which are built up of such units—the cell. Consequently the inference is that[4] *death occurs as the result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony*

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[4] Walton, *Science*, p. 216, 1909.

—*the individual—perish.* The experiments of Woodruff ('11, etc.) who in extending the work of Maupas and of Calkins was able to rear several thousand generations of *Paramecium* without conjugation, as well as the investigations of Harrison subsequently elaborated by Carrel, where human and other animal tissues maintained cell division for a prolonged time in an artificial medium, are here of much interest. In each case the result is brought about by the favorable artificial environment, and it is made more clear that death itself is wholly or in part due to the unfavorable conditions surrounding an organism.

IV. A Working Hypothesis of Evolution

Investigations during the last fifteen years, instead of establishing evolution as the simple process of natural selection conjectured by Darwin and others, have made it evident that the results are due to many factors of much complexity. While the diversity of organisms depends on variation—their inheritance and non-inheritance—it is becoming more and more apparent that the term is too comprehensive and covers variations arising in organisms from causes quite different from one another.

The results reached in the preceding pages indicate the need of extending the older terminology as used by Plate, '13, and others where variations are separated into "somations" or fluctuations induced by the environment and not inherited, and "mutations" or blastovariations arising in the germ plasm and inherited, if a clearer understanding is to be obtained of evolution and its application. Therefore the following scheme is proposed.[5]

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[5] Several interesting groupings of variations have been suggested by Spillman, Baur and others, none of which, however, appear to meet present conditions.

VARIATIONS

A^1. Apparently originating in accordance with definite laws .... A. **Normations.**
  B^1. Induced by general environmental stimuli, (food supply, use and disuse, etc.), but not inherited so far as evidence shows............ 1. *Fluctuations.*
  B^2. Not induced by environmental stimuli; inherited.
    C^1. Arising through the transference of factors by the combination of two ancestral lines in accordance with Mendelian principles, but exhibiting ''per se'' no definite progress...... 2. *Amphimutations* (''mutations'' in part).
    C^2. Arising through causes at present unknown, but which, from the progressive results obtained, may be assumed to originate in accordance with definite laws .... 3. *Cumulations.*[6]
A^2. Apparently not originating in accordance with definite laws ....B. **Abnormations.**
  B^1. Induced during early developmental stages of the embryo from intracellular (?) stimuli, and inherited.
    C^1. Arising through the abnormal segregation of the hereditary material (chromosomes?) .. 4. *Malsegregations* (''mutations'' in part).
    C^2. Arising by the loss of hereditary qualities.
      D^1. Resulting from the functional loss of a factor controlling a character .... 5. *Defactorations* (''mutations'' in part).
      D^2. Resulting from the partial functional loss of a factor controlling a character.... 6. *Fractionations* (''mutations'' in part).
  B^2.. Induced during the early developmental stages of the embryo from extracellular (?) stimuli and not inherited .... 7. *Malformations.*[7]

While any scheme presented must change as new facts are obtained, a terminology is of value in proportion as it gives a basis for future progress. The objection that it is not possible to point out a specific cumulation by no means indicates the absence of such progressive variations taking long intervals of time, by the haphazard method of nature, in which to bring about a change evident to mankind. That the weight of evidence, so far as investigations have gone, is against evolution by means of the other variations noted, makes the explanation the more plausible. While it is true that Bateson ('14) has urged the consideration of the proposition that organic changes occur through the loss of inhibiting factors—defactorations—such a double negative theory assumes a decreasing complexity instead of an increasing complexity of protoplasm, as already pointed out by Castle, ('15) and seems impossible to maintain.

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[6] Cumulations—from cumulo, to increase—including the names of the following groups, with the exception of fractionations proposed by Bateson, are based on the apparent origin of the variations.

[7] Many so-called malformations originate as defactorations, etc.

On the interpretation here presented, the diversity of organic forms is more complex than earlier imagined, and the problem of positive racial improvement is still far from solution. Loss as well as segregation factors may add new forms which really contain nothing new. To build up and not to break down is the desideratum, and the data obtained would seem to suggest that pure line breeding with the employment of statistical methods to show any progress would be the path leading most directly to the goal.

VI. CONCLUSIONS

1. *Direct Conclusions*

The following conclusions drawn from the investigation are primarily statements of fact.

1. Zygospores of *Spirogyra inflata* (Vauch.) produced by lateral conjugation or close breeding (quasi-parthenogenesis) are relatively 26 per cent. more variable in length and 31 per cent. more variable in diameter as measured by the coefficient of variation, than those produced by scalariform conjugation or cross breeding (sexual reproduction).

2. The size (volume) is greater in the average (mean) zygospore close bred by lateral conjugation, where the mean length is 62.38 μ. $\pm$ .178, than in the average zygo-

spore cross bred by scalariform conjugation, where the mean length is 60.44 μ. ± .135, The diameter is approximately the same in both types.

3. In zygospores produced by lateral conjugation there exists a positive correlation between length and diameter of .1894 = .0460, while in scalariform conjugation the value is .0934 = .0473. This is in general agreement with results obtained by others although here the difference is not significant when the probable error is considered.

4. In the material studied approximately 45 per cent. of the zygospores were formed by lateral conjugation, the remaining 55 per cent. by scalariform conjugation.

5. The material studied was strictly homogeneous, and evidently arose from the same parental stock, both types of filaments being intermingled with no structural differences except those of conjugation. Consequently the differences in variability are not the result of fluctuability.

2. *Indirect Conclusions*

The conclusions here presented are generalizations based on the present investigation as well as the work of others, and represent propositions concerning which differences of opinion may exist.

1. Amphimixis, cross-breeding, etc., decreases and does not augment variability (cumulability) although amphimutability may temporarily be increased.

2. Close bred forms are more highly correlated in respect to related characters than cross-bred forms.

3. Variations, so far as their origin is concerned, may be separated into (*A*) Normations consisting of (1) fluctuations, (2) amphimutations, and (3) cumulations, and into (*B*) Abnormations consisting of (1) malsegregations, (2) defactorations, (3) fractionations, and (4) malformations.

4. Cumulations may best be investigated among organisms produced asexually, by pure lines, or by close breeding than by cross breeding, etc.

5. Sexual reproduction and cross fertilization have been advantageous in the evolution of organisms by limiting cumulability and thus confining the progress of the group to a path bounded by the more permanent environment.

6. Death occurs as a result of the continually forming body cells becoming so variable through the absence of control by amphimixis, that eventually some one group fails to meet the limits imposed by the environment, and these together with the remainder of the colony—the individual—perish.

3. *Hypotheses*

The following opinions in the nature of hypotheses based to a large extent on the preceding work may be confirmed or invalidated by future investigations.

1. Variability (cumulability) will be greater in a small and isolated population than in a large and less isolated population.

2. Progressive evolution has resulted from factors arising through cumulations without reference to amphimutations (Mendelian combinations).

3. Characters once established by cumulations produce by fluctuations, amphimutations, etc., the diversity of organic life. Such secondary variations are only indirectly the products of evolution.

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